REORIENTATION OF A LONG-WAVELENGTH CHLOROPHYLL-*a*–PROTEIN BY DIVALENT CATIONS AS REVEALED BY THE LINEAR DICHROISM OF MAGNETO-ORIENTED THYLAKOIDS

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Received 14 March 1978

1. Introduction

There is considerable evidence that low concentrations of divalent cations exert a regulatory role in the light-driven reactions of thylakoids during photosynthetic electron transfer. Mg^{2+} in particular causes increases in the quantum efficiencies of PS II reactions, the magnitude of the variable yield fluorescence and enhancement, and simultaneously decreases the efficiency of PS I reactions [1–16]. These and numerous related observations have been variously interpreted in support of a divalent cation regulation of various parameters that include energy distribution between the two photosystems [1,3,4,8–13,16], the number of PS II fluorescing units [9], the activation of PS II [17], the size of the PS II absorption cross section [7] and modification of the macroscopic organization of thylakoids [5,18–21].

Following a suggestion in [15], Mg^{2+} has been proposed to change the mutual orientation of pigments within the thylakoid, thereby altering the proximity of the photosystems and the rate of energy transfer between them [9,10,14,22].

The involvement of protein–cation interactions in thylakoids have been demonstrated [18] and Ca^{2+} has been shown to bind specifically to the light-harvesting chlorophyll *a*/*b* protein [22]. Support for the requirement of this complex in energy redistribution using immunological methods is in [17]. Conformational changes of membrane protein per se have been inferred from experiments showing that the divalent cation effects are not demonstrable in thylakoids that have been fixed with glutaraldehyde [2,9,11].

This paper presents evidence for a divalent cation-induced change in pigment orientation within the thylakoid in support of the suggestion in [15]. The reorientation of a long-wavelength form of chlorophyll-*a* (Ca_{90}) by Mg^{2+} or Ca^{2+} was observed by the linear dichroism of thylakoids oriented in a magnetic field. In the absence of divalent cations the dichroism of chlorophyll-*a* (Ca_{682}) was confirmed [23,24] and was found to survive fixation by glutaraldehyde. The cation-induced reorientation of Ca_{690} did not occur after fixation, however, suggesting the direct participation of a Ca_{690}–protein complex in the regulation of thylakoid photoreactions.

2. Experimental

Thylakoids were isolated from either pea seedlings or Romaine lettuce and were washed and stored in 100 mM sucrose, 5 mM Tris–Cl, pH 8.0 as in [2].

Absorption and linear dichroism spectra were determined at room temperature using a single beam instrument. The measuring was modulated at 1 kHz and detected by means of a 2.54 cm diameter photodiode (United Dectector Technology, PIN 25) placed 3 cm from the sample and immediately behind an opal glass diffuser to minimize scattering losses. After current to voltage conversion the transmission signal was amplified and demodulated using a lock-in amplifier (Princeton Applied Research, Model 126).

A film linear polarizer (Oriel Optics, Model 2734) was inserted into the optical path immediately before the sample of thylakoids which were magnetically oriented at a field strength of 18 kG [25] using an electromagnet (Spectromagnetic Industries, Model 6001). Previous studies have shown that the thylakoids orient perpendicularly to the magnetic field [23,24]. Thus, in the configuration we employed, the plane of the membranes was parallel to the optical path.

Absolute spectra (A) were recorded with the electric vector of the measuring light either vertical or horizontal to give $A_{\parallel}$ and $A_{\perp}$, respectively. Linear dichroism spectra (ΔA) were obtained by subtraction ($A_{\parallel} - A_{\perp}$) at 0.5 nm intervals.

3. Results

Figures 1 and 2 show examples of room temperature absorption and linear dichroism spectra in the red wavelength region of thylakoids isolated from higher plants. The wavelength at maximum absorption is 678 nm, and the absorption band is considerably 'flattened' due to scattering [26]. The addition of 3.3 mM $MgCl_2$ (not shown) resulted in increased scattering and further optical flattening but the absorption maximum was not shifted.

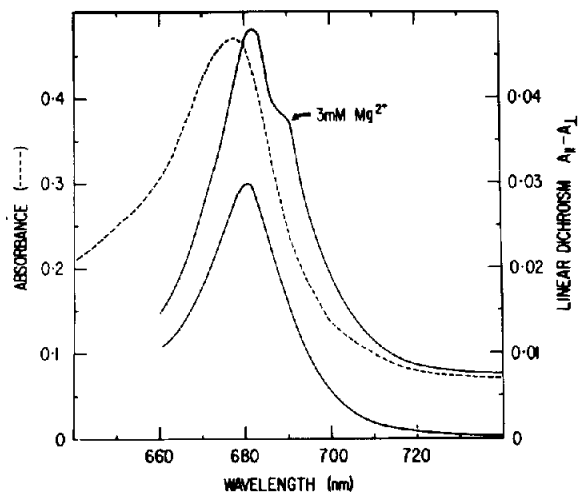

Fig.1. Room temperature absorption and linear dichroism spectra of pea thylakoids. The linear dichroism spectra $A_{\parallel} - A_{\perp}$ (————) were determined with thylakoids oriented in a magnetic field of 18 kG and the absolute absorption spectrum (- - - - -) was determined at zero field. The thylakoids were suspended in 100 mM sucrose, 5 mM Tris–Cl, pH 8.0 and $MgCl_2$ was added to give final conc. 3.3 mM, where indicated.

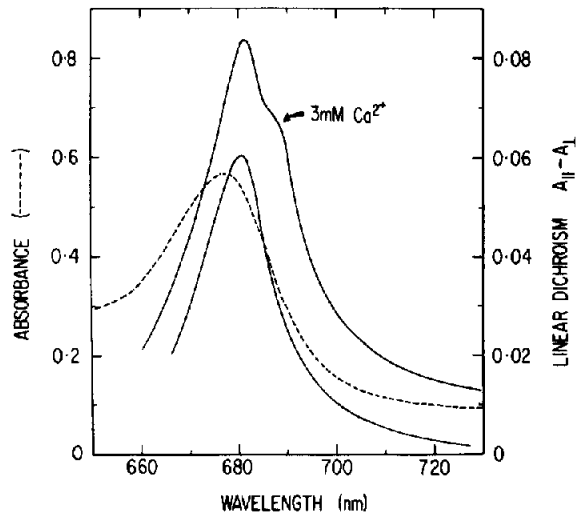

Fig.2. Room temperature absorption (- - - - -) and linear dichroism spectra (————) of lettuce thylakoids. Conditions as in fig.1. $CaCl_2$ was added to give final conc. 3.3 mM.

In the absence of divalent cations the linear dichroism spectra of magneto-oriented thylakoids were positive with a maximum at 682 nm and an 18 nm bandwidth. Lower peak linear dichroism values ($\Delta A/A$) were observed in aged preparations, and no ΔA signals were detected in sonicated thylakoids or those that had been extensively swollen in hypotonic buffer (5 mM Tris–Cl, pH 8.0). The data are in agreement with [23,24] although the magnitude of the dichroism ($\Delta A/A$) was not as great. The linear dichroism spectrum in this wavelength region has been interpreted [23,24] as indicating that the transition moments of long-wavelength chlorophyll-*a* forms have a high degree of orientation in the plane of the thylakoid membrane.

Figures 1 and 2 also show the effect of low concentrations of divalent cations on the linear dichroism spectra of thylakoids. It can be seen that both Mg^{2+} and Ca^{2+} modify the spectra, cause an increase in signal amplitude and a very pronounced long-wavelength asymmetry of the ΔA band. Under these conditions the ΔA maximum was usually at 682 nm or

slightly longer and the shoulder at about 687–695 nm. The bandwidth also increased from 18–27 nm. This modification in linear dichroism by divalent cations was prevented by inclusion of EDTA (10 mM) and was not observed in spectra of thylakoids fixed with 0.05% glutaraldehyde. However, the dichroism observed in the absence of divalent cations persisted and was not altered by fixation of the membranes.

The changes in the linear dichroism spectra were found to be specific for divalent cations. No changes in ΔA were detected upon addition of monovalent cations (K^+ or Na^+) although substantial increases in light scattering were observed.

The linear dichroism spectra show that the orientation of the chlorophyll-*a* forms at 682 nm are not altered by the addition of Mg^{2+} or Ca^{2+}, but the appearance of a long-wavelength shoulder and the increased width of the ΔA band strongly suggest that the divalent cations induce a reorientation of another long-wavelength component of chlorophyll-*a* into the plane of the thylakoid. The difference between the linear dichroism spectra of the thylakoids in the presence and absence of Mg^{2+} or Ca^{2+} reveals a pigment with a maximum dichroism at 690 nm and a 20 nm bandwidth. The amplitude of this increment in ΔA indicating the reoriented chlorophyll-*a* was somewhat variable between preparation and species and in some instances was as high as 75% of the Ca_{682} band.

4. Discussion

These data indicate that divalent cations interact with the thylakoid and cause reorientation of a long-wavelength form of chlorophyll-*a* into the plane of the membrane. The addition of divalent cations also increased scattering and optimal flattening of the absorption band. It might be argued that such effects alone were responsible for the change in the linear dichroism spectrum. However, the possible contribution of artifacts that might invalidate such measurements was assessed [23,24] and it was concluded that effects due to flattening, textural dichroism and polarized selective scattering and reflection may be neglected. These conclusions were reinforced by studies on the electric dichroism of chloroplast fragments and pigment–protein complexes [27].

The concentration of divalent cations required to reorient the long-wavelength component is similar to that required to modify the quantum efficiency of PS I and PS II reactions [1,6,7,10,12] and to increase the variable component of fluorescence [2–7,8–11, 13,14,16]. Taken with the apparent specificity for the divalent cations this suggests a direct relationship. It is, therefore, likely that the conformational change in the thylakoid as reflected by this pigment reorientation is intimately involved in the regulation of the photoacts.

The red absorption band of chlorophyll in vivo has been analyzed [28,29] and multiple forms including one that absorbs at 690 nm have been shown. This long-wavelength form is found only in CP I [29,30], a chlorophyll-*a*–*P*-700–protein complex, that is postulated to be derived from PS I [31]. We would suggest that this form of chlorophyll-*a* is involved in the divalent cation-induced pigment reorientation in the thylakoid and we expect additional components will be identified upon further analysis.

The change in dichroism of Ca_{690} was not observed in thylakoids treated with glutaraldehyde and, therefore, the functional amino groups of protein must participate in the pigment reorientation. Accordingly we propose that divalent cations cause a reorientation of a Ca_{690}–protein complex within the thylakoid and this change in conformation is directly concerned with the regulation of the photoreactions.

Acknowledgements

This work was supported by funds from the National Science Foundation (BMS-74-19700 and PCM 77-07694) and Department of Energy (contract EY-76-S-02-2932). We thank Susan R. Quinones for assistance with some of the experiments and Drs W. S. Shipp and A. W. Holowinsky for valuable advice. We are grateful to Dr P. H. Rieger of the Department of Chemistry for the loan of the electromagnet.

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